TETRAHYDROISOQUINOLINE-AND TETRAHYDROBENZAZEPINE DERIVATIVES AS IGF-1R INHIBITORS

FIELD OF THE INVENTION

The present invention relates to novel compounds capable of down-regulating or inhibiting the expression or function of the insulin-like growth factor-1 receptor (IGF-1R). The invention is also directed to methods of down-regulating or inhibiting IGF-1R expression or function in order to prevent and/or treat cancer and other abnormal cell growth, and metabolic as well as blood vessel proliferate disorders, in which uncontrolled expression of this receptor is observed.

BACKGROUND OF THE INVENTION

The insulin-like growth factor receptor (IGF-1R) is one of 58 trans-membrane tyrosine kinase receptors present in humans [Review: Structure and function of the Type 1 insulin-like growth factor receptor. T.E.Adams et al. Cell. Mol. Life Sci. 57 (2000) 1050-1093]. Genetic evidence and studies on cells lacking the IGF-1 receptor have demonstrated that it is required for optimal growth, but not an absolute condition for growth [Baserga et al. Biochim. Biophys. Acta 1332(1997) 105-126]. An expression of the IGF-1 receptor protects cells from apoptosis and seems to be a requirement for the establishment and maintenance of the transformed phenotype both in vitro and in vivo [R. Baserga et al. Biochim. Biophys. Acta 1332 (1997) 105-126]. Several *in vitro* and *in vivo* studies have demonstrated that inhibition of the expression or function of the IGF-1 receptor reverses the transformed phenotype and inhibits tumor cell growth. The techniques used in these studies include neutralizing antibodies [Kalebic et al. Can-

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cer Res. 54(1994) 5531-5534], antisense oligonucleotides
[Resnicoff *et al.* Cancer Res. 54(1994) 2218-2222], dominant
negative receptors [D'Ambrosio *et al.* Cancer Res. 56(1996)
4013-4020], triple-helix forming oligonucleotides [Rinnin-
5   sland *et al.* Proc.Natl. Acad. Sci. 94(1997) 5854-5859], anti-
sense mRNA [Nakamura *et al.* Cancer Res. 60(2000) 760-765] and
RNA interference using a double stranded RNA (V.M. Macaulay
et al. WO-A-03/100059).

The use of antisense oligonucleotides to inhibit the
10 IGF-1 receptor expression in keratinocytes has been shown to
reverse the epidermal hyper proliferation in psoriasis le-
sions [C.J. Wraight *et al.* Nat. Biotechnol. 18(2000) 521-
526].

Down-regulation of the IGF-1 receptor would possibly
15 also have beneficial effect with respect to diseases such as
diabetic retinopathy [L.K. Shawver *et al.* DDT 2(1997) 50-63]
as well as atherosclerosis and restenosis [A.Bayes-Genis *et
al.* Circ. Res. 86(2000) 125-130].

The IGF-1 receptor system is regarded as an attractive
20 target in the prevention and/or treatment of diseases that
are dependant on an expression or over-expression of the IGF-
1 receptor for their proliferation [L. Long *et al.* Cancer Re-
search 55(1995) 1006-1009, R. Baserga TIBTECH 14(1996) 150-
152; R. Baserga *et al.* Endocrine 7 (August 1997) 99-102; V.M.
25 Macaulay *et al.* Annals of Oncogene 20 (2001) 4029-4040].

PRIOR ART

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A series of substances, named tyrphostins, have been claimed to down-regulate or inhibit the expression of the IGF-1 receptor [M. Parrizas et al. Endocrinology 138 (1997) 1427-1433; G. Blum et al. Biochemistry 39(2000) 15705-15712; G. Blum et al. J. Biol. Chem. 278 (2003) 40442-40454]. The drawback with the tyrphostins are their low activity in cell systems and that they cross-react with the insulin receptor.

It has been demonstrated [L. Kanter-Lewensohn et al. Mol. Cell. Endocrinology 165 (2000) 131-137] that tamoxifen, at high concentration, has the ability to down-regulate or inhibit the tyrosine phosphorylation of the IGF-1R β-subunit, thereby blocking downstream signalling.

In US patent 6337338 B1, a number of heteroaryl-aryl urea substances are described as antagonists of the IGF-1 receptor. In cell growth inhibition studies on MCF-7 and MCF-10 cell lines the substances showed low activities.

In the patent publication WO 02/102804 A1 it is demonstrated that podophyllotoxin, deoxypodophyllotoxin, picropodophyllin and deoxypicropodophyllin are selective and efficient inhibitors of the IGF-1 receptor. Deoxypicropodophyllin has previously [A. Akahori et al. Chem. Pharm. Bull. 20(1972) 1150-1155] been shown to be superior to deoxypodophyllotoxin in retarding the death of mice inoculated with lymphatic leukemia L1210. No mechanism of action, however, was proposed.

In the patent publication WO 02/102805 A1 it is shown that also acetylpodophyllotoxin, epipodophyllotoxin, podophyllotoxone and 4'-demethylpodophyllotoxin are potent inhibitors of the IGF-1R phosphorylation.

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In the patent publication WO 03/048133 Al a number of pyrimidine derivatives are described as modulators of the IGF-1 receptor.

The present invention aims to provide compounds with improved IGF-1R down-regulating activity.

SUMMARY OF THE INVENTION

The object set is achieved by the compounds of the following formula (I):
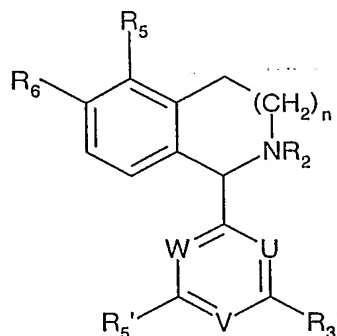
(I)

wherein

R$_2$ designates hydrogen, Me, Et, CHO, CN, OH, OMe, COR$_9$, COOR$_9$, CONHR$_9$ or CSNHR$_9$, whereby R$_9$ denotes (C$_1$-C$_4$)alkyl;

R$_5$ designates hydrogen, (C$_1$-C$_4$)alkyl, OH, (C$_1$-C$_4$)alkoxy, OCF$_3$, trifluoromethyl or halogen;

R$_6$ designates Me, (C$_1$-C$_4$)alkoxy, OCF$_3$, SMe or SEt;

n is 1 or 2;

R$_3$' and R$_5$' each independently designate OH, Me, Et, OMe, OCF$_3$, trifluoromethyl or halogen;

U designates N or CR$_2$', whereby R$_2$' denotes hydrogen, (C$_1$-C$_4$)alkyl, (C$_1$-C$_4$)alkoxy, trifluoromethyl or halogen;

V designates N or CR_4', whereby R_4' denotes hydrogen, (C_1-C_6)alkoxy, (C_1-C_6)alkyl, OH, trifluoromethyl or halogen;

W designates N or CR_6', whereby R_6' denotes hydrogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, trifluoromethyl or halogen;

and pharmaceutically acceptable salts thereof, where applicable (see below).

Preferred embodiments of the compound (I) are derivable from the dependent claims. The most preferred examples of compounds of formula (I) are those of claim 13.

Further objects of the invention are the use of the compounds (I) as a medicament, particularly for the prevention or treatment of diseases in which the down-regulation or inhibition of the expression or function of the IGF-1 receptor is considered beneficial, and pharmaceutical compositions containing a compound (I).

DETAILED DESCRIPTION OF THE INVENTION

The compounds of formula (I) contain a tetrahydroisoquinoline moiety (n = 1) or a tetrahydrobenzazepine moiety (n = 2).

In the above formula (I) preferably R_2 is Me, OH, CN, CHO, COR_9 or $COOR_9$; Particularly preferred examples of R_2 are Me (methyl), CHO (formyl), COMe (acetyl) and CN (cyano).

Preferably R_5 is hydrogen, Me, OMe or halogen; and preferably R_6 is OMe or OEt. Particularly preferably R_5 is hydrogen or OMe and R_6 is OMe. The most preferred substituent pattern for R_5 and R_6 is R_5 = hydrogen and R_6 = OMe.

In formula (I) the substituent on the 1-position of
the 1,2,3,4-tetrahydroisoquinoline or 2,3,4,5-tetrahydro-1H-
2-benzazepine moieties may be a phenyl substituent (U = CR_2';
V = CR_4'; W = CR_6',), a 4-pyridyl substituent (U = CR_2'; V =
N; W = CR_6'), a 2-pyridyl substituent (V = CR_4'; U = N, W =
CR_6', or U = CR_2', W = N), a 2-pyrimidyl substituent (U, W =
N; V = CR_4'), a 4-pyrimidyl substituent (V = N; U = CR_2', W =
N, or U = N, W = CR_6'), or a triazinyl substituent (U, V, W =
N).

A preferred substitution pattern on said substituent
on the 1-position is R_3', R_5' = each independently chloro,
bromo, Me or OMe. In one more preferred embodiment R_3' and
R_5' are identical. In another preferred embodiment they are
both chloro, both bromo, both Me or both OMe; in another
preferred embodiment R_3' is chloro or bromo, and R_5' is OMe.
Most preferably both R_3' and R_5' are chloro or bromo. When
the 1-substituent is phenyl then R_2' and R_6' are preferably
hydrogen. R_4' then is preferably hydrogen, chloro, bromo, Me
or OMe. Three most preferred substitution patterns on the
phenyl as the 1-substituent are a) R_3', R_4', R_5' = OMe; b) R_3'
= chloro, R_4', R_5' = OMe; and c) R_4' = hydrogen and R_3' and
R_5' = both chloro or both bromo. Due to the rotational
freedom of the phenyl, in b) the definitions for R_3' and R_5'
are interchangeable.

The alkyl residue in the (C_1-C_4)alkyl or (C_1-C_4)alkoxy,
as used in the substituent definitions of formula (I), may be
branched, unbranched or cyclic and may contain double or tri-
ple bonds. It is e.g. methyl, ethyl, n-propyl, n-butyl, iso-
propyl, sec-butyl, t-butyl, cyclopropyl, cyclobutyl, ethenyl,

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prop-2-enyl or prop-3-enyl, but-1-enyl, but-2-enyl, but-3-enyl or propargyl. Preferably it is methyl, ethyl or isopropyl; particularly preferably it is methyl.

The alkyl residue in the (C_1-C_6)alkyl or (C_1-C_6)alkoxy may be unbranched, branched or cyclic and may contain double or triple bonds. Examples of unbranched alkyls are methyl, ethyl, n-propyl, n-butyl, n-pentyl and n-hexyl. Examples of branched alkyl are isopropyl, sec-butyl, t-butyl, (1,1-diethyl)methyl, (1-propyl-1-methyl)methyl, (1-isopropyl-1-methyl)methyl, (1,1-dimethyl-1-ethyl)methyl, (1-t-butyl)methyl, (1-propyl-1-ethyl)methyl, (1-isopropyl-1-ethyl)methyl, (1,1-diethyl-1-methyl)methyl and (1-t-butyl-1-methyl)methyl. Examples of the cyclic alkyl are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or (2- or 3-methyl)cyclopentyl. Examples of unsaturated alkyls are ethenyl, prop-2-enyl, but-1-enyl, but-2-enyl, but-3-enyl, pent-1-enyl, pent-2-enyl, pent-3-enyl, pent-4-enyl, penta-1,3-dienyl, penta-1,4-dienyl, penta-2,4-dienyl or propargyl.

The term "halogen" means in the context of the present application fluoro, chloro or bromo.

In the context of the present application the term "IGF-1 receptor" encompasses human IGF-1 receptor, the amino acid sequence of which is known [see e.g. T.E. Adams et al. Cellular and Molecular Life Sciences 2000, 57, p. 1050-1093], but it also encompasses other IGF-1R, such as IGF-1R of mammals in general.

The pharmaceutically acceptable salts of the compounds of formula (I) are acid addition salts with pharmaceutically acceptable acids, which are possible in the case where R_2 is hydrogen, Me or Et; and/or at least one of U, V and W is nitrogen. Examples of pharmaceutically acceptable acids are hydrochloric, hydrobromic, methanesulfonic, acetic, propionic, benzoic, citric, tartaric, malic, maleic, fumaric, lactic, nitric, phosphoric or succinic acid.

The compounds (I) of the present invention can be prepared using the methods described below, with reference to schemes 1a and 1b. Preferably the compounds (I) of the present invention are synthesized *via* the imine (II), which is a 3,4-dihydroisoquinoline (n=1) or a 4,5-dihydro-3H-2-benzazepine (n=2). The imine (II) may then be converted by reduction to a secondary amino compound (I) of the invention, where R_2 = hydrogen. As the reducing agent sodium borohydride in methanol or other reducing agents may be used, such as DI-BAL, B_2H_6, $LiAlH_4$, or catalytic hydrogenation using a catalyst, which may be chiral, suited for reducing imines, but which will not influence other parts of the compound (I, R_2 = hydrogen).

The compounds (I) where R_2 = Me or Et and the 1-substituent is phenyl, may be prepared by alkylation of (II) with a corresponding alkyl halide R_2X, where X is a leaving group such as bromo, iodo, mesylate, tosylate or triflate, to form an intermediate iminium salt (III) (scheme 1a). This alkylation is preferably performed at room temperature to reflux temperature, in an aprotic solvent such as acetone, DMF, CH_3CN, DMSO or 1,2-dimethoxyethane. The iminium salt (III) is then reduced under similar conditions as above for the reduc-

tion of the imine (II) itself, to form compounds (I) of the invention where R_2 = Me or Et.

The compounds (I) where R_2 = Me or Et, respectively, and the 1-substituent is other than phenyl (i.e. at least one of U, V or W is nitrogen) may be prepared by acylation of (I), where R_2 = hydrogen, with XCOOEt or XCOMe, respectively, where X is a leaving group such as chloro (in XCOMe X may also be acetoxy), to form a compound (I) wherein R_2 is COOEt or COMe, respectively, which is then reduced, e.g. with LiAlH$_4$ or B_2H_6, to the compound (I) with R_2 = Me or Et, respectively (scheme 1b).

For all compounds (I), where R_2 = methyl, the standard Eschweiler-Clarke reaction may also be used, to directly form these derivatives from the corresponding secondary amino compound (I) with R_2 = hydrogen (scheme 1a or 1b).

All compounds (I) where R_2 = COR$_9$, COOR$_9$ or cyano can be prepared from the above secondary amino compound (I), where R_2 = hydrogen, by acylation with an appropriate acyl halide R$_9$COX, (in particular for R$_9$ = Me also acetic anhydride may be used), haloformic acid ester R$_9$OCOX, or cyanogen halide XCN (X = chloro or bromo); by using a suited auxiliary base such as NEt$_3$ or pyridine and, optionally, a catalyst such as 4-dimethylaminopyridine (schemes 1a or 1b). The reaction temperature may be from room temperature to the boiling temperature of the solvent, which solvent may be an ether such as THF or 1,2-dimethoxyethane; CH$_3$CN; N-methylpyrrolidone or CH$_2$Cl$_2$. In the case a cyanogen halide is used, anhydrous potassium carbonate may be used to neutralize the formed hydrogen halide. Acetylations (R_2 = COMe) are prefera-

bly performed in neat acetic anhydride, i.e. without solvent
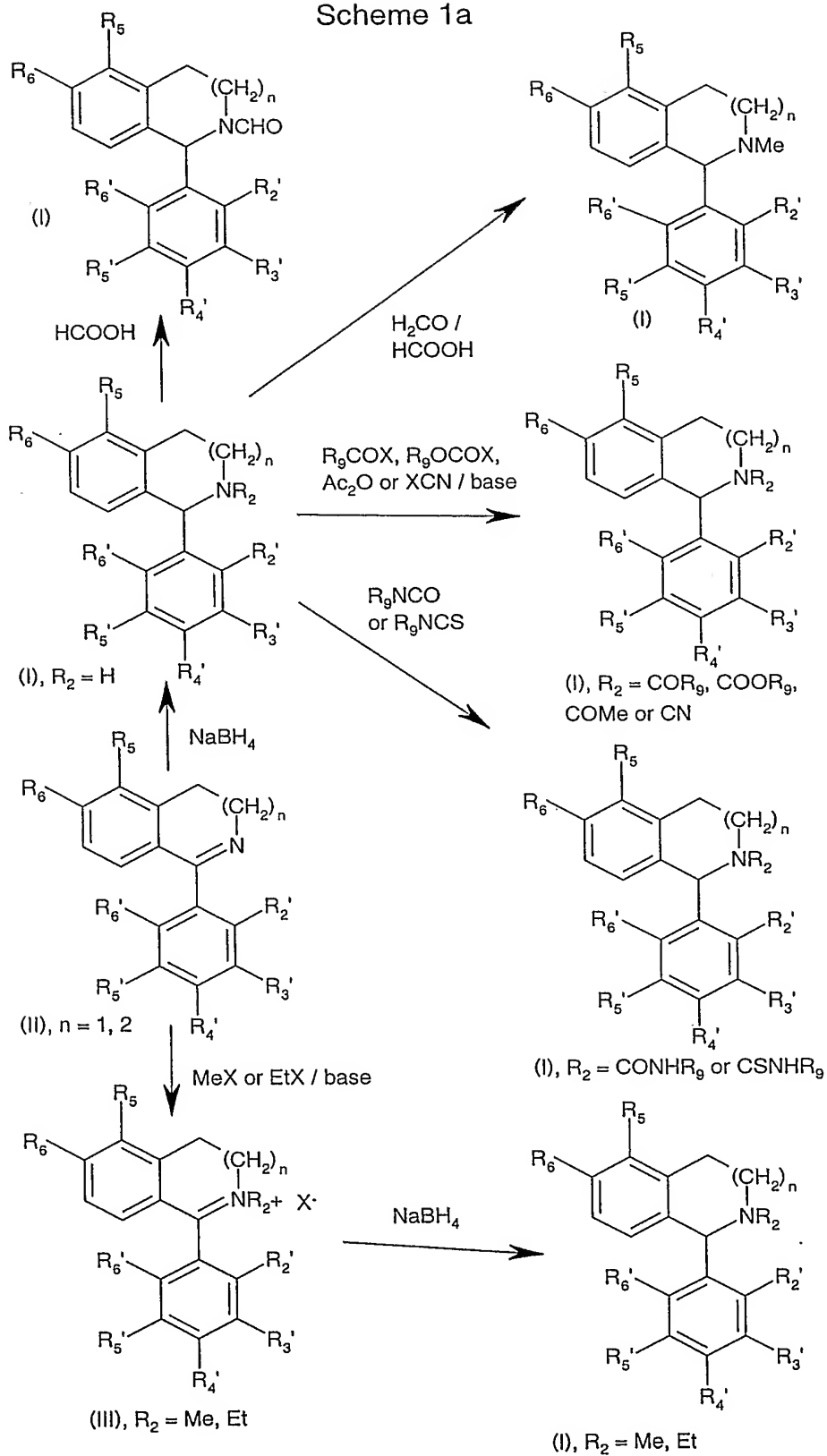or catalysts, to facilitate the isolation of the N-acetyl de-
rivative.

All compounds (I) where R_2 = formyl, may be prepared
from the above secondary amino compound (I), where R_2 = hy-
drogen, by using formic acid in refluxing toluene (schemes 1a
and 1b).

All compounds (I) where R_2 = CONHR$_9$ or CSNHR$_9$ can be
prepared from a secondary amino compound (I), where R_2 = hy-
drogen, under standard condition by reacting this with an
isocyanate OCNR$_9$ or isothiocyanate SCNHR$_9$ at room temperature
in an inert solvent such as an ether, DMF or acetonitril
(schemes 1a and 1b).

The imine (II) itself may be prepared from an appropriately
substituted phenetylamine (VIII) or 3-phenylpropylamine (IX)
(scheme 2), by acylating either of these with an appropri-
ately substituted acyl chloride (X), e.g. under standard
Schotten-Baumann conditions, which gives an amide (IV). This
amide (IV) may then be cyclized to the imine (II) under dehy-
drating conditions with a dehydrating agent such as zinc
chloride or POCl$_3$ (Bischler-Napieralski type) or P_2O_5 (Pictet-
Gams type).

Either of the amines (VIII) or (IX) may be prepared by
techniques known in the art from appropriately substituted
benzaldehydes (V). For the sequence (V) to (VI) to (VIII)
reference is made e.g. to Kohno et al., Bull. Chem. Soc.
Jpn., 1990, 63(4), 1252-1254. In some cases the phenethyl
amine (VIII) is even commercially available, as is the case

Scheme 1a

Scheme 1b

(II), n = 1, 2

NaBH$_4$

H$_2$CO / HCOOH

(I)

(I), R$_2$ = H

R$_9$COX, R$_9$OCOX,
Ac$_2$O or XCN / base

R$_9$NCO
or R$_9$NCS

(I), R$_2$ = COR$_9$, COOR$_9$,
COMe or CN

XCOOEt, XCOMe,
or Ac$_2$O / base

(I), R$_2$ = COOEt or COMe

(I), R$_2$ = CONHR$_9$ or CSNHR$_9$

HCOOH

LiAlH$_4$ or B$_2$H$_6$

(I), R$_2$ = Me or Et

(I)

Scheme 2

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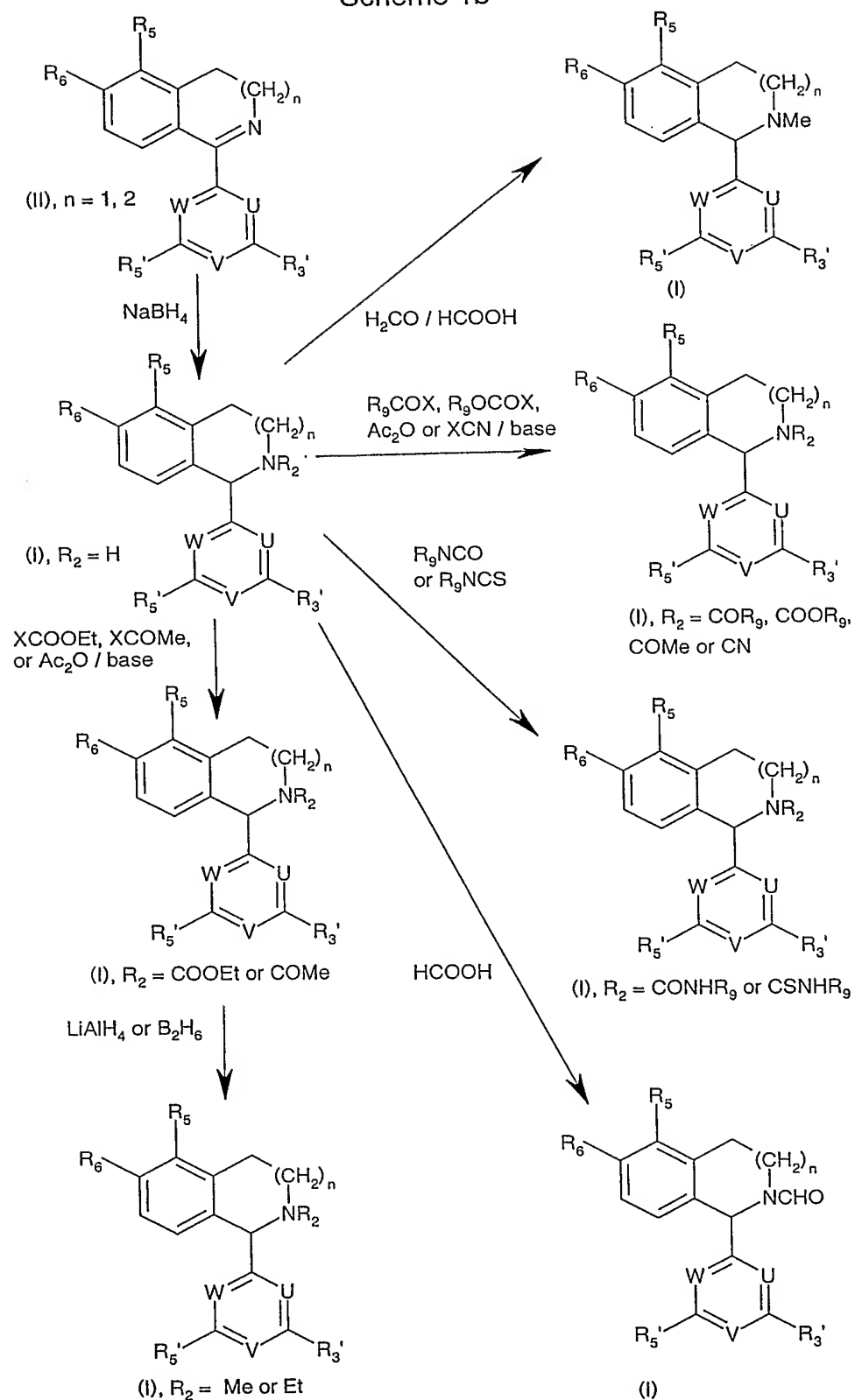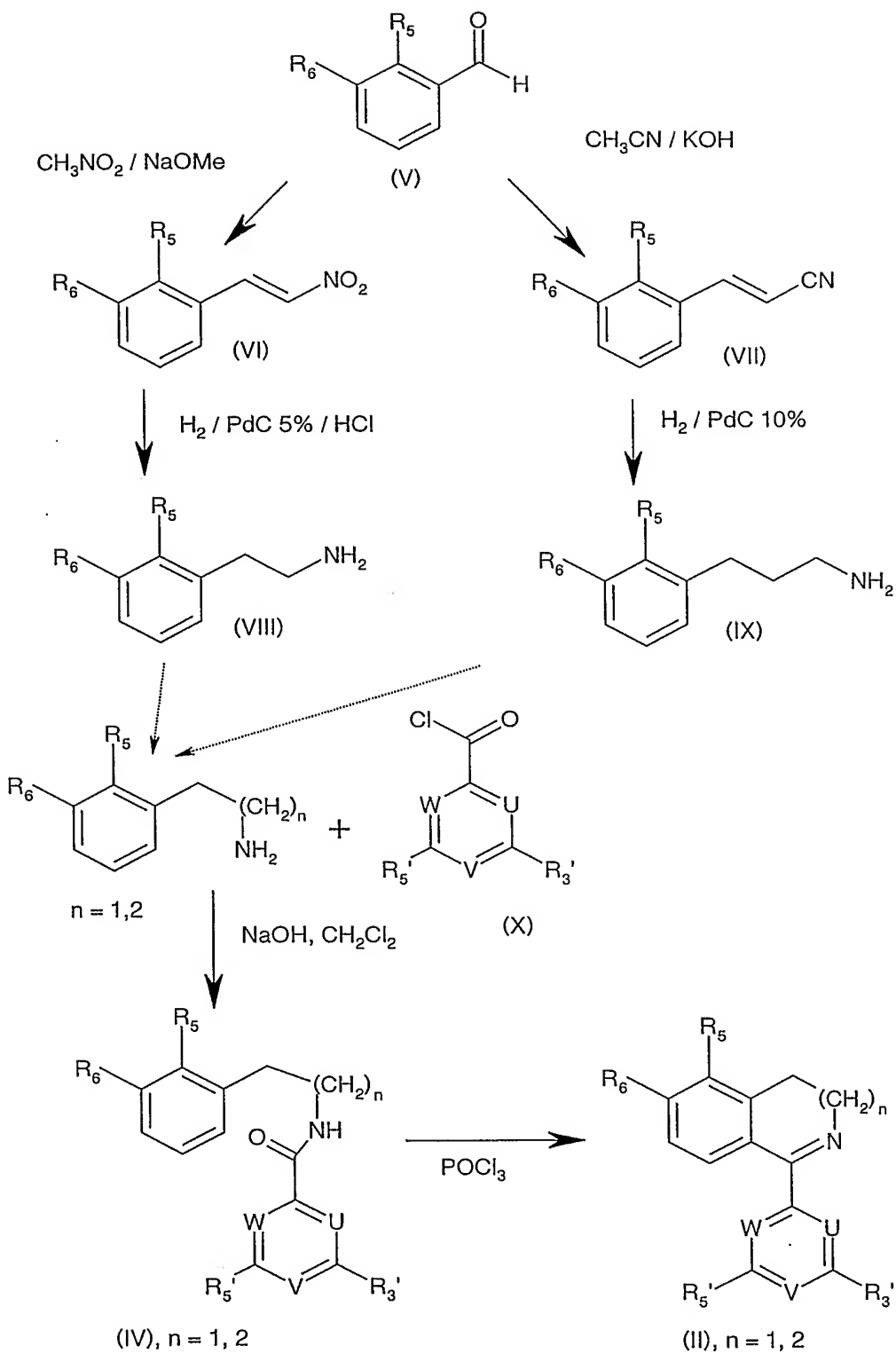
for 3-methoxyphenylethylamine, which *inter alia* was used in examples 31-38 (see below). The sequence (V) to (VII) is easily achieved in accordance with the procedure devised for the corresponding 4-methoxy derivative (DiBiase, S.A. et al. J.Org.Chem. 44(1979) 4640-4649). Compound (VII) is there-after reduced to the amine (IX) by catalytic hydrogenation. The appropriately substituted benzaldehyde (V) of scheme 2 in turn is either commercially available or known from the literature.

Some examples of known benzaldehydes (V) that may be used for synthesizing some preferred compounds (I) are the following:

benzaldehyde (V)	CAS reg. no.
3-methoxybenzaldehyde	591-31-1
2-fluoro-3-methoxybenzaldehyde	103438-88-6
2-chloro-3-methoxybenzaldehyde	54881-49-1
2-bromo-3-methoxybenzaldehyde	10401-18-0
2-hydroxy-3-methoxybenzaldehyde	148-53-8
3-ethoxybenzaldehyde	22924-15-8
2-chloro-3-ethoxybenzaldehyde	99586-82-0
3-ethoxy-2-hydroxybenzaldehyde	492-88-6
2-chloro-3-methylbenzaldehyde	61563-28-8
2-bromo-3-methylbenzaldehyde	109179-31-9
3-isopropoxybenzaldehyde	75792-33-5
2-hydroxy-3-propyloxybenzaldehyde	222031-84-7
3-butyloxy-2-hydroxybenzaldehyde	91849-57-9
2-hydroxy-3-isobutyloxybenzaldehyde	222031-85-8
2-hydroxy-3-isopropoxybenzaldehyde	222031-87-0
3-methylbenzaldehyde	620-23-5
2-hydroxy-3-methylbenzaldehyde	824-42-0
2,3-dimethoxybenzaldehyde	86-51-1
2,3-diethoxybenzaldehyde	24454-82-8
2-ethoxy-3-methoxybenzaldehyde	66799-97-1
3-ethoxy-2-methoxybenzaldehyde	75792-34-6
3-isopropoxy-2-methoxybenzaldehyde	218903-24-3
2-methoxy-3-methylbenzaldehyde	67639-61-6
2-ethoxy-3-methylbenzaldehyde	532965-62-1
3-methoxy-2-methylbenzaldehyde	56724-03-9
3-hydroxy-2-ethylbenzaldehyde	532966-36-2

3-methoxy-2-propylbenzaldehyde	97582-12-2
2-isopropyl-3-methoxybenzaldehyde	93351-17-8
2-butyl-3-methoxybenzaldehyde	151038-64-1
2-(1,1-dimethylethyl)-3-methoxybenzaldehyde	151038-66-3
3-(trifluoromethoxy)benzaldehyde	52771-21-8
3-hydroxy-2-methoxybenzaldehyde	66495-88-3
3-hydroxy-2-ethoxybenzaldehyde	182067-51-2
3-hydroxy-2-propoxybenzaldehyde	508202-83-3
3-(methylthio)benzaldehyde	73771-35-4
3-(ethylthio)benzaldehyde	87425-00-1
3-bromo-2-fluorobenzaldehyde	149947-15-9
2-fluoro-3-hydroxybenzaldehyde	103438-86-4
2-chloro-3-hydroxybenzaldehyde	56962-10-8
2-bromo-3-hydroxybenzaldehyde	196081-71-7
3-hydroxybenzaldehyde	100-83-4
3-hydroxy-2-methylbenzaldehyde	90111-15-2
3-hydroxy-2-propylbenzaldehyde	532966-38-4
3-hydroxy-2-isopropylbenzaldehyde	532966-40-8
2-butyl-3-hydroxybenzaldehyde	532966-42-0
2-(1,1-dimethylethyl)-3-hydroxybenzaldehyde	532966-46-4
3-hydroxy-2-(1-methylpropyl)benzaldehyde	532966-44-2
2-hydroxy-3-trifluoromethoxybenzaldehyde	497959-31-6
2-hydroxy-3-(methylthio)benzaldehyde	67868-82-0
3-benzyloxy-2-hydroxybenzaldehyde	86734-59-0

2-(C_1-C_4)alkyl-3-(C_1-C_4)alkoxybenzaldehydes (i.e. with R_5 = (C_1-C_4)alkyl, R_6 = (C_1-C_4)alkoxy) and 2-(C_1-C_4)alkyl-3-trifluoromethoxy-benzaldehydes (i.e. with R_5 = (C_1-C_4)alkyl, R_6 = OCF$_3$), respectively, may be synthesized from 2-(C_1-C_4)alkyl-3-hydroxy-benzaldehydes by Williamson etherification with a corresponding (C_1-C_4)alkyl bromide and trifluoromethyliodide, respectively.

2-(C_1-C_4)alkoxy-3-(C_1-C_4)alkoxybenzaldehydes (i.e. with R_5 = (C_1-C_4)alkoxy, R_6 = (C_1-C_4)alkoxy) and 2-(C_1-C_4)alkoxy-3-trifluoromethoxy-benzaldehydes (i.e. with R_5 = (C_1-C_4)alkoxy, R_6 = OCF$_3$), respectively, may be synthesized from 2-(C_1-C_4)alkoxy-3-hydroxy-benzaldehydes by Williamson etherification with a corresponding (C_1-C_4)alkyl bromide and trifluoromethyliodide, respectively. Alternatively all these compounds are available from 3-benzyloxy-2-hydroxybenzaldehyde by

etherification, followed by debenzylation and etherification of the 3-hydroxy group.

2-(C_1-C_4)alkyl-3-methylthio-benzaldehydes (i.e. with R_5 = (C_1-C_4)alkyl, R_6 = SMe) and 2-(C_1-C_4)alkyl-3-ethylthio-benzaldehydes (i.e. with R_5 = (C_1-C_4)alkyl, R_6 = SEt), respectively, may be synthesized from 2-(C_1-C_4)alkyl-3-bromo-benzaldehyde diethyl acetals by reacting its Grignard reagent with dimethyl sulfide or diethyl sulfide, respectively (for a similar reaction see M. Euerby et al., Synthetic Communications 11 (1981), 849-851).

2-(C_1-C_4)alkoxy-3-methylthio-benzaldehydes (i.e. with R_5 = (C_1-C_4)alkoxy, R_6 = SMe) and 2-(C_1-C_4)alkoxy-3-ethylthio-benzaldehydes (i.e. with R_5 = (C_1-C_4)alkoxy, R_6 = SEt), respectively, may be synthesized from 2-(C_1-C_4)alkoxy-3-bromo-benzaldehydes by reacting its Grignard reagent with dimethyl sulfide or diethyl sulfide, respectively (for a similar reaction see M. Euerby et al., Synthetic Communications 11 (1981), 849-851). Another route to these starting materials is by etherification of 2-hydroxy-3-(methylthio)benzaldehyde or 2-hydroxy-3-(ethylthio)benzaldehyde (A. Makoto *et al.* Bull. Chem. Soc. Jpn. 51 (1978) 2435-2436).

The appropriately substituted acyl chlorides (X) for the synthesis of the amide (IV) are benzoyl chlorides, when U = CR_2', V = CR_4' and W = CR_6'; and are known or can be synthesized under standard conditions from corresponding benzoic acids with thionyl chloride or oxalyl chloride. Some examples of known benzoyl chlorides (X) and benzoic acids that may be used for synthesizing some preferred compounds (I) are the following:

benzoyl chloride (X)	CAS No.
3,5-difluorobenzoyl chloride	129714-97-2
3,5-dichlorobenzoyl chloride	2905-62-6
3,5-dibromobenzoyl chloride	23950-59-6
3,5-diethylbenzoyl chloride	57664-62-7
3,5-dimethoxybenzoyl chloride	17213-57-9
3,5-dimethylbenzoyl chloride	6613-44-1
3,5-bis(trifluoromethyl)benzoyl chloride	785-56-8
3-bromo-5-chlorobenzoyl chloride	21900-27-6
3-chloro-5-methylbenzoyl chloride	21900-22-1
3-methoxy-5-methylbenzoyl chloride	96227-40-6
3-bromo-5-methoxybenzoyl chloride	157893-14-6
3-chloro-5-methoxybenzoyl chloride	89106-53-6
3-fluoro-5-(trifluoromethyl)benzoyl chloride	171243-30-4
3,4,5-trimethoxybenzoyl chloride	4521-61-3
3,4,5-trifluorobenzoyl chloride	177787-26-7
3,4,5-trichlorobenzoyl chloride	42221-50-1
3,4,5-trimethylbenzoyl chloride	57498-46-1
4-bromo-3,5-dimethoxybenzoyl chloride	56518-43-5
4-chloro-3,5-dimethoxybenzoyl chloride	56518-47-9
3,5-dimethoxy-4-methylbenzoyl chloride	34523-76-7
3,5-dibromo-4-methoxybenzoyl chloride	4073-36-3
3,5-dichloro-4-methoxybenzoyl chloride	29568-76-1
3,5-dichloro-4-methylbenzoyl chloride	113485-46-4
3,5-dimethyl-4-methoxybenzoyl chloride	21668-34-8
3,5-difluoro-4-methoxybenzoyl chloride	501701-43-5
3,5-difluoro-4-methylbenzoyl chloride	103877-74-3
3-bromo-4,5-dimethoxybenzoyl chloride	70574-46-8
3,4-dichloro-5-methoxybenzoyl chloride	63001-38-7
3,5-diethyl-4-methoxybenzoyl chloride	59931-54-3
3,5-dibromo-4-fluorobenzoyl chloride	402-85-7
2,3,5-trimethoxybenzoyl chloride	119098-79-2
5-bromo-2,3-dimethoxybenzoyl chloride	107188-91-0
3,5-dichloro-4-isopropoxybenzoyl chloride	41490-23-7

Benzoic acid	Cas No.
3-chloro-4,5-dimethoxybenzoic acid	20624-87-7
3,5-dimethoxy-4-isopropoxybenzoic acid	52009-58-2
3,5-dihydroxy-4-methoxybenzoic acid	4319-02-2
3,4-dihydroxy-5-methoxybenzoic acid	3934-84-7
3-chloro-4-hydroxy-5-methoxybenzoic acid	62936-23-6
3,5-dimethoxy-4-hydroxybenzoic acid	329320-56-1
3,5-dichloro-4-hydroxybenzoic acid	112290-09-2
3-bromo-5-chloro-4-hydroxybenzoic acid	118276-15-6
3-bromo-4-hydroxy-5-methoxybenzoic acid	6324-52-3
3-chloro-4-hydroxy-5-methylbenzoic acid	35458-34-5

Appropriately substituted benzoic acids are known or
may easily be synthesized by using standard procedures as
known by those skilled in the art. It will be appreciated by
those skilled in the art that in processes of the present in-
vention certain functional groups such as hydroxyl groups in
the starting reagents or intermediate compounds may need to
be protected by protecting groups. Thus, the preparation of
the compounds (I) may involve the addition and removal of one
or more protecting groups. The protection and deprotection of
functional groups is described in "Protective Groups in Or-
ganic Chemistry", edited by J.W.F. McOmie, Plenum Press
(1973) and "Protective Groups in Organic Synthesis", 2nd edi-
tion, T.W. Greene and P.G.M. Wuts, Wiley-Interscience (1991).

Suitable protecting groups for aromatic hydroxyl groups
in the present invention are *e.g.* benzyl or isopropyl groups.
Removal of the benzyl group and the isopropyl group is easily
achieved by catalytic hydrogenation (catalyst Pd/carbon) and
treatment with BCl_3, respectively.

The appropriately substituted acyl chlorides (X),
where U = CR_2', V = N and W = CR_6', can be synthesized under
standard conditions from appropriately substituted isonico-
tinic acids with thionyl chloride. The appropriately sub-
stituted acyl chlorides (X), where U = N, V = CR_4' and W =
CR_6' can be synthesized under standard conditions from 2-
carboxylic acid substituted pyridines. The appropriately
substituted acyl chlorides (X), where U = N, V = CR_4' and W
= CR_6', or where U = CR_2', V = CR_4' and W = N; can be syn-
thesized under standard conditions from appropriate 2-car-
boxylic acid substituted pyridines. The appropriately sub-
stituted acyl chorides (X), where U = CR_2' and V, W = N, can

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be synthesized under standard conditions from appropriate 4-carboxylic acid substituted pyrimidines. The appropriately substituted acyl chlorides (X), where U, W = N and V = CR$_4$' can be synthesized under standard conditions from appropriate substituted 2-carboxylic acid substituted pyrimidines. The appropriately substituted acyl chlorides (X), when U, V, W = N, can be substituted under standard conditions from appropriate 2-carboxylic acid substituted triazines.

Some examples of suitable starting materials for the production of nitrogen containing acid chlorides (X) are the following known compounds:

acid chloride (X)	CAS No.
4,6-dimethoxypyrimidine-2-carbonyl chloride	509101-33-1

Starting materials for production of (X)	CAS No.
2,6-dichloro-4-pyridinecarboxylic acid	5398-44-7
2-chloro-6-methoxy-4-pyridinecarboxylic acid	15855-06-84
4,6-dichloro-2-pyridinecarboxylic acid	88912-25-8
4,6-dimethoxy-2-pyridinecarboxylic acid	90764-84-4
2,6-dichloro-4-pyrimidinecarboxylic acid	16492-28-7
4,6-dichloro-1,3,5-triazine-2-carboxamide	583630-76-6
4,6-dimethyl-1,3,5-triazine-2-ethylcarboxylate	829-73-2
4,6-dimethoxy-1,3,5-triazine-2-carboxaldehyde	98141-06-1

The transformation of amides, ethyl esters and aldehydes into their corresponding carboxylic acid derivatives are well-known reactions for those skilled in the art.

The compounds of the present invention contain a chiral center and therefore may exist in different enantiomeric forms. Although particularly preferred compounds (I) are enantiomerically pure the scope of the present invention

is intended to cover both enantiomers *per se,* as well as mixtures of them in any ratio, such as racemic mixtures.

Compounds (I) of the present invention may be obtained in their enantiomerically pure forms by crystallization of their addition salts with chiral acids [see *e.g.* D.L. Minor *et al.* J. Med. Chem. 37 (1994) 4317-4328; US patent 4349472], or alternatively, may be isolated by preparative HPLC using commercially available chiral phases. Other routes to the pure enantiomers of the products of the present invention are the use of asymmetric synthesis [M.J. Munchhof *et al.* J. Org. Chem. 60(1995) 7086-7087; R.P. Polniaszek *et al.* Tetrahedron Letters 28 (1987) 4511-4514], by asymmetric transfer hydrogenation of the intermediate imines (II) or iminium salts (III) [N. Uematsu *et al.* J. Am. Chem. Soc. 118 (1996) 4916-4917; G.. Meuzelaar *et al.* Eur. J. Org. Chem. 1999, 2315-2321], or by resolution of chiral diastereometric derivatives thereof, as known by those skilled in the art.

The compounds of formula (I) and their pharmaceutically acceptable salts, where applicable, may be administered in the form of a pharmaceutical composition in which they are in association with a pharmaceutically acceptable adjuvant, diluent or carrier, in order to prevent or treat any disease in which inhibition of the IGF-1 receptor would be considered beneficial by the skilled person. The present invention also provides a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, as hereinbefore defined, in association with a pharmaceutically acceptable adjuvant, diluent or carrier. As to the appropriate excipients, diluents and adjuvants, reference may be made to the standard literature describing these, e.g.

to chapter 25.2 of Vol. 5 of "Comprehensive Medicinal Chemis-
try", Pergamon Press 1990, and to "Lexikon der Hilfsstoffe
für Pharmazie, Kosmetik und angrenzende Gebiete", by H.P.
Fiedler, Editio Cantor, 2002 (in German).

5   The compounds (I) of the examples of the present in-
vention have IC_{50} activities in intact cell systems ranging
from 8 microgram/ml to 150 picogram/ml. Due to the large dif-
ference in activities, the pharmaceutical compositions of the
invention will preferably comprise from 0.001 to 50 % by
10   weight of compound (I).

The daily dose of compounds (I) will necessarily be
varied depending upon the host treated, the particular route
of administration, and the severity and kind of the illness
being treated. Accordingly the optimum dosage may be determi-
15   ned by the practitioner who is treating any particular pati-
ent.

The pharmaceutical compositions of the invention may
be formulated as creams, gels, solutions, ointments, suspen-
sions or plasters *etc.* when intended for topical administra-
20   tion; for administration by inhalation, e.g. as aerosols or
dry powders; for oral administration, e.g. in the form of
tablets, capsules, gels, syrups, suspensions, solutions, pow-
ders or granules; for rectal or vaginal administration *e.g.*
as suppositories; or for parenteral injection (including in-
25   travenous, subcutaneous, intramuscular, intravascular, or in-
fusion) as a sterile solution, suspension or emulsion.

The compounds of the present invention were found to
down-regulate or inhibit the expression or function of the

human IGF-1 receptor, without inhibiting the structurally closely related insulin receptor. They were found to promote apoptosis of malignant cells and to interfere with cell divi- sion by blocking the cells in the prophase of the mitotic cy-

5   cle. The compounds (I) are useful for the prevention and/or treatment of diseases of unregulated IGF-1R expression, in- cluding cell proliferate diseases such as cancer, atheroscle- rosis, restenosis, inflammatory diseases e.g. psoriasis, autoimmune diseases e.g. rheumatoid arthritis, and transplant

10 rejection. Some examples of cancers in which IGF-1R is unregulated or overexpressed and which can be prevented and/or treated by the compounds (I) of the invention include, but are not limited to, cancer of the breast, prostate, colon, lung, brain, pancreas, and melanoma, multiple myeloma,

15 lymphoma and leukemia. Under the paragraph "Biological Data" are described some techniques to evaluate the sensitivity of cancer cells towards compounds (I) of the invention and the presence of the IGF-1 receptor.

     Optionally the compounds (I) may be used against cell

20 proliferate diseases in combination with conventional treat- ments such as irradiation and/or one or more chemotherapeutic agents such as e.g. Actinomycin, Altretamine, Bleomycin, Busulphan, Capecitabine, Carboplatin, Carmustine, Chloram- bucil, Cisplatin, Cladribine, Crisantaspase, Cyclophosphamid,

25 Cytarabine, Dacarbazine, Daunorubicin, Doxorubicin, Epirubi- cin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Ida- rubicin, Ifosfamide, Irinotecan, Lomustine, Melphalan, Mer- captopurine, Methotrexate, Mitomycin, Mitoxantrone, Ox- aliplati, Pentostatin, Procarbazine, Streptozocin, Taxol, Te-

30 mozolomide, Thiotepa, Tioguanine/Thioguanine, Topotecan,

Treosulfan, Vinblastine, Vincristine, Vindesine or Vinorelbine.

When a chemotherapeutic agent is used in combination with the compound of formula (I), then this may be used in the form of a medicament containing a combination of these two agents, for simultaneous administration, or they may be used in the form of separate dosage forms, each containing one of the agents, and in the latter case the individual dosage forms may be used e.g. sequentially, i.e. one dosage form with the compound (I), followed by a dosage form containing the chemotherapeutic agent (or vice versa). This embodiment of two separate dosage forms may be conceived and provided in the form of a kit.

In addition to their use in therapeutic medicine, the compounds (I) and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardization of in vitro and in vivo test systems for the evaluation of the effects of inhibitors of cell cycle activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

EXAMPLES

Products described in the Examples have satisfactory proton nuclear magnetic resonance spectra and/or mass spectral data. Melting points are uncorrected. The substances described in the examples are racemates, unless marked with (-), which denotes the levorotatory enantiomer.

Examples 1 to 30: syntheses of racemic compounds (I)

In the examples 1 to 30 the following general synthetic procedures were used:

1. Production of amides (Scheme 2, IV):

5   The appropriate amine (VIII or IX, 0.1 mol) was added
to an aqueous solution of sodium hydroxide (200 ml, 2M) and
dichloromethane (200 ml). To the vigorously stirred mixture
containing the amine, the appropriate acyl chloride (X, 0.1
mol) dissolved in dichloromethane (200 ml) was added during
10   30 minutes at room temperature. After the addition, the mix-
ture was stirred for further 60 minutes. The dichloromethane
phase was separated, washed with hydrochloric acid (200 ml,
2M), dried (sodium sulphate) and concentrated to dryness. The
residual amide (IV) is suitable without further purification
15   as a starting material for the production of imines. All
produced amides that were obtained in a crystalline state
could be re-crystallized from methanol.

2. Production of imines (Scheme 2, II):

       A mixture of the appropriate amide (IV, 0.05-0.1 mol),
20   toluene (200 ml) and phosphorus oxychloride (80 ml) was re-
fluxed for 1.5-24 hours. The progress of the reaction was
followed by TLC (silica gel/ethyl acetate or methanol). The
reaction mixture was concentrated to dryness and partitioned
between ethyl acetate (500 ml) and aqueous sodium hydroxide
25   (400 ml, 2 M). The formed imine (II) was transferred into an
aqueous phase by extraction of the organic phase with hydro-
chloric acid (3 x 200 ml, 2 M), which was made alkaline (pH

11-12) and extracted with dichloromethane. The organic phase
was dried and concentrated to dryness giving the imine. If
needed, most imines (II) could be purified by crystallization
from diethyl ether or ethanol, or by crystallization of the
corresponding hydrochlorides from ethanol.

3. Production of secondary amino compounds (I) by re-
duction of imines (Schemes 1a and 1b):

A solution of the appropriate imine (II, 0.0 1-0.05
mol) in methanol (200 ml) was treated with an excess of so-
dium borohydride at room temperature until no starting mate-
rial remained. The mixture was concentrated to dryness and
partitioned between aqueous sodium hydroxide (300 ml, 2M) and
dichloromethane (400 ml). The organic phase was separated,
dried and concentrated to dryness leaving the pure secondary
amine. The amino compound (I, R_2 = hydrogen) could be crys-
tallized from diethyl ether or ethanol, or by crystallization
of the corresponding hydrochloride from ethanol or etha-
nol/diethyl ether.

4. Production of N-alkyl compounds (Scheme 1a, III and
I; R_2 = Me or Et):

The appropriate imine (II, 0.005-0.01 mol) was dis-
solved in acetone (25-50 ml) and the selected alkyl halide
MeX or EtX (1.2 equivalents) was added. The mixture was
stirred at room temperature or at reflux temperature for 1-24
hours, depending upon the nature of the alkyl halide. After
cooling to room temperature, the formed iminium salt (III)
was filtered off and dried. The iminium salt so obtained was
treated as described for the reduction of imines under para-

graph 3 above. The compounds (I), with R_2 = Me or Et, were crystallized from diethyl ether or ethanol, or by crystallization of the corresponding hydrochlorides from ethanol or ethanol/diethyl ether.

N-methyl compounds (I) could also be produced by Eschweiler-Clarke reaction. A mixture of the appropriate secondary amino compound (I, R_2 = hydrogen, 0.005-0.01 mol), 1,2-dimethoxyethane (10 ml), formaldehyde (37 % in water, 5 ml) and formic acid (5 ml) was heated at 80°C for 5 hours. The reaction mixture was concentrated to dryness and the N-methyl compound (I) was isolated as described for secondary amino compounds (I) under paragraph 3.

5. Production of N-acetyl compounds (Scheme 1b, I; R_2 = COMe):

The appropriate secondary amino compound (I, 0.005-0.01 mol) was treated with acetic anhydride (150 ml) at room temperature during 24 hours. The mixture was concentrated to dryness leaving the N-acetyl compound (I), which was crystallized from methanol (except products from examples 6, 8, and 51 which were obtained as gums, and from examples 43, 44 and 47, which were isolated as amorphous solids).

6. Production of N-formyl compounds (I, Schemes 1a and 1b):

A mixture of the appropriate secondary amino compound (I, 0.005-0.01 mol), formic acid (10 equivalents) and toluene (100 ml) was heated under reflux for 18 hours using a Dean-Stark trap. The reaction mixture was concentrated to dryness

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and the residue was dissolved in ethyl acetate. The organic phase was washed with 2M hydrochloric acid, dried and concentrated to dryness leaving the N-formyl compound (I).

7. Production of N-acyl compounds (I, Schemes 1a and 1b):

A mixture of the appropriate secondary amino compound (I, 0.005-0.01 mol), pyridine (25 ml) and the selected acyl chloride R_9COCl (1.2 equivalents) was heated at 80°C for two hours. The reaction mixture was concentrated to dryness and partitioned between ethyl acetate and 2M sodium hydroxide. The organic phase was washed with 2M hydrochloric acid, dried and concentrated to dryness, leaving the N-acyl compound (I).

8. Production of N-carboxylic acid ester compounds (I, Schemes 1a and 1b):

A mixture of the appropriate secondary amino compound (I, 0.005-0.01 mol), anhydrous potassium carbonate (5 equivalents), acetone (100 ml) and the selected chloroformate R_9OCOCl (2 equivalents) was refluxed for 24 hours. The reaction mixture was concentrated to dryness, and the residue partitioned between hydrochloric acid (100 ml, 2 M) and dichloromethane (300 ml). The organic phase was dried and concentrated to dryness, giving the N-carboxylic acid ester compound (I).

9. Production of N-carboxylic acid amide compounds I and N-carbothioic acid amide compounds (I, Schemes 1a and 1b):

The appropriate secondary amino compound (I, 0.005-
0.01 mol) was dissolved in acetonitrile (25 ml) and treated
with the selected isocyanate OCNR$_9$ or isothiocyanate SCNR$_9$ (2
equivalents) at room temperature for 24 hours. The mixture

5   was concentrated to dryness and the residue crystallized from
methanol, giving the title compound (I).

10. Production of N-cyano compounds (I, Schemes 1a and
1b):

A mixture of the appropriate secondary amino compound

10   (I, 0.005-0.01 mol), 1,2-dimethoxyethane (10 ml), dry sodium
carbonate (5 equivalents) and cyanogen halide, such as cyano-
gen bromide (2 equivalents) was heated at 50°C for three
hours. The reaction mixture was partitioned between dichloro-
methane (200 ml) and 2M hydrochloric acid (100 ml). The or-

15   ganic phase was dried and concentrated to dryness, leaving
the N-cyano compound (I), which was crystallized from metha-
nol.

By appropriate use of the above outlined general syn-
thesis steps 1-10 racemic compounds (I) according to the fol-

20   lowing table 1 were prepared. Melting points given in the ta-
ble are uncorrected.

Table 1

Ex.	compound (I)	appearance	crystallization solvent	m.p.
1	1-(3,5-dichlorophenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	134-137°C
2	1-(3,5-dibromophenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	170-173°C
3	1-(3,4,5-trimethoxyphenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	165-169°C
4	1-(3,5-dimethoxyphenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	152-154°C
5	1-(3,5-dimethylphenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	ethanol	120-122°C
6	1-[3,5-di(trifluoromethyl)phenyl]-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	viscous oil	--	--
7	1-(2,6-dichloro-4-pyridyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	163-166°C
8	1-(3,5-difluorophenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	viscous oil	--	--
9	1-(3,4,5-trimethoxyphenyl)-2-acetyl-6-ethoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	138-140°C
10	1-(3,4,5-trimethoxyphenyl)-2-acetyl-5,6-dimethoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	166-168°C
11	1-(3,4,5-trimethoxyphenyl)-2-acetyl-6-methyl-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	176-178°C
12	1-(3,4,5-trimethoxyphenyl)-2-formyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	103-105°C
13	1-(3,4,5-trimethoxyphenyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	94-95°C

14	1-(3,4,5-trimethoxyphenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	acetone	118-120°C.
15	1-(3,5-dichlorophenyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	diethyl ether	112-115°C
16	1-(3,5-dichlorophenyl)-2-ethyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride	white solid	ethanol/diethyl ether	195-199°C
17	1-(3,5-dimethoxyphenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-carbothioic acid ethyl amide	white solid	methanol	149-151°C
18	1-(3,5-dimethoxyphenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid ethyl amide	white solid	methanol	142-144°C
19	1-(3,5-dimethoxyphenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid methyl ester	white solid :	methanol	87-89°C
20	1-(3,4,5-trimethoxyphenyl)-2-acetyl-6-isopropoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	109-111°C
21	1-(3,5-dichlorophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	diethyl ether	122-124°C
22	1-(2,3-dimethoxy-5-bromophenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	pale yellow amorphous solid	--	--
23	1-(3,4,5-trimethoxyphenyl)-2-cyano-6-ethoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	137-139°C
24	1-(3-chloro-4,5-dimethoxyphenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	methanol	173-175°C
25	1-(3-chloro-4-isopropoxy-5-methoxy)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	White solid	methanol	117-119°C
26	1-(3-chloro-4-hydroxy-5-methoxy)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	White solid	methanol	189-191°C
27	1-(2,6-dichloro-4-pyridyl)-2-formyl-6-	white solid	methanol	138-140°C

	methoxy-1,2,3,4-tetrahydroisoquinoline			
28	1-(3-chloro-4-acetyloxyoxy-5-methoxy)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	ethanol	205-215°C (dec)
29	1-(3-chloro-4-cyclopentyloxy-5-methoxy)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	white solid	ethanol	228-232°C
30	1-(3,5-dichlorophenyl)-2-methyl-7-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepine	white solid	diethyl ether	121-123°C
31	1-(3,4,5-trimethoxyphenyl)-2-methyl-7-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepine hydrochloride	white solid	diethyl ether	165-167°C
32	1-(3,4,5-trimethoxyphenyl)-7-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepine	white solid	diethyl ether	127-129°C

Examples 33-40: syntheses of enantiomerically pure compounds (I)

Example 33: (-)-1-(3,4,5-trimethoxyphenyl)-2-cyano-6-methoxy-1,2,3,4-tetrahydroisoquinoline

5   1.   3-Methoxyphenylethylamine (25.0 g) was added to an aqueous solution of sodium hydroxide (200 ml, 2M) and dichloromethane (200 ml). To the vigorously stirred mixture containing the amine, 3,4,5-trimethoxybenzoyl chloride (38.1 g) dissolved in dichloromethane (200 ml) was added during 30

10   minutes at room temperature. After the addition, the mixture was stirred for further .60 minutes. The dichloromethane phase was separated, washed with hydrochloric acid (200 ml, 2M), dried (sodium sulphate) and concentrated to dryness. The residual amide (57.2 g) is suitable without further purifica-

15   tion as a starting material for the production of the corresponding imine. An analytical sample was obtained by crystallization from methanol, giving a white solid, m.p. 115-117°C.

       2.   A mixture of the amide from step 1 (52.0 g), toluene (350 ml) and phosphorus oxychloride (140 ml) was heated

20   under reflux for 1.5 hours. The reaction mixture was concentrated to dryness and partitioned between ethyl acetate (500 ml) and aqueous sodium hydroxide (400 ml, 2 M). The formed imine was transferred into an aqueous phase by extraction of the organic phase with hydrochloric acid (3 x 300 ml, 2 M),

25   which was made alkaline (pH 11-12) and extracted with dichloromethane. The organic phase was dried and concentrated to dryness giving the imine, (48.2 g). An analytical sample

was obtained by crystallization from methanol giving a white
solid, m.p. 141-143°C.

3. The imine produced according to step 2 (69.3 g) was
dissolved in a mixture of methanol (500 ml) and 1,2-dimeth-
oxyethane (300 ml) and treated with sodium borohydride at
room temperature until no starting material remained (TLC:
silica gel/methanol). The mixture was concentrated to dryness
and partitioned between aqueous sodium hydroxide (500 ml, 2M)
and dichloromethane (500 ml). The organic phase was sepa-
rated, dried and concentrated to dryness, leaving the secon-
dary amine (67.8 g). An analytical sample was obtained by
crystallization from ethyl acetate, giving a white solid,
m.p. 118-120°C.

4. The secondary amine (48.3 g) produced according to
step 3 was dissolved in hot ethanol (600 ml) and the solution
was added to acetyl-D-leucine (25.0 g) dissolved in hot etha-
nol (200 ml). The mixture was allowed to reach room tempera-
ture during 24 hours, after which it was filtered. The re-
tained crystals were washed with ethanol (200 ml) and dried
giving a white solid (60.0 g, 10.9 % ee). A second crystalli-
zation (59.7 g) from ethanol (1400 ml) gave a white solid
(39.2 g, 37.9 % ee). A third crystallization (39.0 g) from
ethanol (1150 ml) gave a white solid (26.0 g, 77.2 % ee). A
fourth crystallization (25.7 g) from ethanol (900 ml) gave a
white solid (21.6 g, 99.9 % ee). The product from the last
crystallization was partioned between dichloromethane (400
ml) and aqueous sodium hydroxide (400 ml, 2M). The organic
phase was dried and concentrated to dryness, leaving the (-)
enantiomer (13.9 g). Crystallization from ethanol gave the
pure (-) enantiomer (12.4 g, 100.0 % ee). The corresponding

hydrochloride, crystallized from methanol, was used for char-
acterization purposes, m.p. 270-275°C (dec.), $[\alpha]_D^{20}$ -46.8°
(c = 0.051, DMF).

5. A mixture of the pure enantiomer (0.50 g) from step
4, 1,2-dimethoxyethane (20 ml), dry sodium carbonate (0.30 g)
and cyanogen bromide (0.35 g) was heated at 50EC for three
hours. The reaction mixture was partioned between dichloro-
methane (200 ml) and hydrochloric acid (100 ml, 2M). The or-
ganic phase was dried and concentrated to dryness. The resi-
due was crystallized from methanol giving the title compound
as a white solid (0.36 g), m.p.132-134°C, $[\alpha]_D^{20}$ -93.2° (c =
1.0, CHCl$_3$).

Example 34: (-)-1-(3,5-dichlorophenyl)-2-acetyl-6-
methoxy-1,2,3,4-tetrahydroisoquinoline

1. 3-Methoxyphenylethylamine (18.1 g) was added to an
aqueous solution of sodium hydroxide (200 ml, 2M) and di-
chloromethane (200 ml). To the vigorously stirred mixture
containing the amine, 3,5-dichlorobenzoyl chloride (25.0 g)
dissolved in dichloromethane (200 ml) was added during 30
minutes at room temperature. After the addition, the mixture
was stirred for further 60 minutes. The dichloromethane phase
was separated, washed with hydrochloric acid (200 ml, 2M),
dried (sodium sulphate) and concentrated to dryness. The re-
sidual amide (40.6 g) is suitable without further purifica-
tion as a starting material for the production of the corre-
sponding imine. An analytical sample was obtained by crystal-
lization from methanol, giving a white solid, m.p. 111-113°C.

2. A mixture of the amide from step 1 (35.8 g), toluene (200 ml) and phosphorus oxychloride (80 ml) was heated under reflux for 6 hours. The reaction mixture was concentrated to dryness and partitioned between ethyl acetate (500 ml) and aqueous sodium hydroxide (400 ml, 2 M). The ethyl acetate phase was dried and concentrated to dryness. The residue was crystallized from methanol, giving the imine (24.0 g), m.p. 110-113°C.

3. The imine from step 2 (18.2 g) was dissolved in methanol (300 ml) containing 1.05 equivalents of acetic acid and treated with an excess of sodium cyanoborohydride at room temperature until no starting material remained (TLC: silica gel-ethyl acetate). The mixture was concentrated to dryness and partitioned between aqueous sodium hydroxide (300 ml, 2M) and dichloromethane (400 ml). The organic phase was separated, dried and concentrated to dryness leaving the secondary amine (17.7 g). An analytical sample was obtained by crystallization from ethanol, m.p. 122-124°C.

4. The secondary amine (46.0 g) produced according to step 3 was dissolved in hot ethanol (800 ml) and the solution was added to N-acetyl-D-leucine (25.84 g) dissolved in hot ethanol (650 ml). The mixture was allowed to reach room temperature during the night, after which it was filtered. The retained crystals were washed with ethanol (150 ml) and thereafter partioned between dichloromethane (500 ml) and aqueous sodium hydroxide (400 ml, 2M). The organic phase was dried and concentrated to dryness, leaving the levorotatory enantiomer (6.9 g, 99.3 % ee). Crystallization from ethanol gave the pure (-)-enantiomer (5.2 g), m.p. 94-95°C, $[\alpha]_D^{20}$ - 24.8° (c = 1.5, $CHCl_3$).

5. The (-)-enantiomer from step 4 (1.6 g) was treated
with acetic anhydride (100 ml) at room temperature during 24
hours. The mixture was concentrated to dryness and the resi-
due was partioned between dichloromethane (200 ml) and hydro-
chloric acid (2M, 100 ml). The organic phase was dried and
concentrated to dryness, leaving the title compound as a
white amorphous solid, $[\alpha]_D^{20}$ -154.9° (c=1.52, CHCl$_3$).

Example 35: (-)-1-(2,6-dichloro-4-pyridyl)-2-formyl-6-
methoxy-1,2,3,4-tetrahydroisoquinoline

1. A mixture of 2,6-dichloroisonicotinic acid (26.1
g), thionyl chloride (140 ml) and 1,2-dimethoxyethane (70 ml)
was refluxed for 6 hours. The excess of thionyl chloride and
solvent were evaporated leaving the acid chloride.

3-Methoxyphenylethylamine (20.6 g) was added to an
aqueous solution of sodium hydroxide (300 ml, 2M) and di-
chloromethane (400 ml). To the vigorously stirred mixture
containing the amine, the acid chloride from above, dissolved
in 1,2-dimethoxyethane (50 ml), was added during 30 minutes
at room temperature. After the addition, the mixture was
stirred for further 60 minutes. The dichloromethane phase was
separated, dried and concentrated to dryness. The residual
amide was crystallized from methanol, giving a white solid
(31.6 g), m.p. 105-108°C.

2. A mixture of the amide produced according to step 1
(38.0 g), toluene (300 ml) and phosphorus oxychloride (80 ml)
was heated under reflux for 5 hours. The reaction mixture was
concentrated to dryness and partitioned between ethyl acetate
(500 ml) and aqueous sodium hydroxide (400 ml, 2 M). The

formed imine was transferred into an aqueous phase by extrac-
tion of the organic phase with hydrochloric acid (5 x 300 ml,
2 M), which was made alkaline (pH 11-12) and extracted with
dichloromethane. The organic phase was dried and concentrated
5   to dryness giving the crude imine (27.3 g). Crystallization
from methanol gave the imine (22.8 g). An analytical sample
was obtained by re-crystallization from acetone, giving a
white solid, m.p. 130-133°C.

    3. A mixture of benzeneruthenium(II) chloride dimer
10   (19 mg), (-)-(1S,2S)-N-(naphthalene-1-sulfonyl)-1,2-di-
phenylethylenediamine (31 mg) [G.J. Meuzelaar et al.
Eur.J.Org.Chem.(1999) 2315-2321], triethylamine (0.5 ml) and
acetonitrile was heated with stirring under nitrogen at 80°C
for one hour. After cooling to room temperature, the imine
15   from step 2 (4.0 g) dissolved in acetonitrile (10 ml) and an
azeotropic mixture of formic acid and triethylamine (10 ml,
5:2) were added to the mixture containing the catalyst. After
20 hours of reaction, the same amount of catalyst and
azeotropic mixture were added to the reaction mixture. After
20   a total reaction time of 47 hours, the reaction mixture was
partioned between aqueous sodium hydroxide (250 ml, 1M) and
ethyl acetate. The organic phase was dried and concentrated
to dryness. The residue was purified by chromatography on
silica gel (40-63 μM, 6x21 cm) using ethyl acetate as eluent.
25   The fraction containing the secondary amine was concentrated
to dryness. The residual amine was transferred into its hy-
drochloric salt by treatment with hydrogen chloride in metha-
nol (1.25 M, 15 ml). Crystallization from methanol afforded
the amine hydrochloride (0.72 g, 99.8 % ee), m.p. 221-260°C
30   (dec.), $[\alpha]_D^{20}$ -28.9° (c=0.72, DMF).

4. A mixture of the free amine from step 3 (0.60 g), formic acid (2 ml) and toluene (100 ml) was heated under reflux for 18 hours using a Dean-Stark trap. The reaction mixture was concentrated to dryness and the residue was dissolved in ethyl acetate (200 ml), which was washed with aqueous sodium hydroxide (100 ml, 2M), dried and concentrated to dryness leaving the formyl derivative as a solid. Crystallization from methanol gave the title compound as a white solid (0.52 g, 100.0 % ee), m.p. 156-158°C, $[\alpha]_D^{20}$ -213.1° (c= 1.05, CHCl$_3$).

Examples 36-40: syntheses of additionally five enantiomerically pure compounds (I)

The enantiomeric pure compounds 34, 36 and 37 were synthesized from the enantiomerically pure secondary amine described in Example 32, step 4, by using the using the above outlined general synthesis steps 4-10. Compound 38 was synthesized from the enantiomerically pure secondary amine described in Example 31, step 4, by using the general synthesis step 6. Compound 35 was synthesized by reduction of 1-(3,5-dimethoxyphenyl)-6-methoxy-3,4-dihydroisoquinoline by asymmetric transfer hydrogenation in accordance with Example 33, step 3, followed by crystallization of the hydrochloride of the formed secondary amine from ethanol. The enantiomerically pure secondary amine was transferred into its formyl derivative by applying step 6 of the general synthesis description. The properties of compounds 34-38 are described in the following Table 2:

Table 2

36	(-)-1-(3,5-dichlorophenyl)-2-formyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline $[\alpha]_D^{20}$ = -206.7° (c = 1.01, CHCl₃)	white amorphous solid	--	--
37	(-)-1-(3,5-dimethoxyphenyl)-2-formyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline $[\alpha]_D^{20}$ = -223.2° (c = 1.04, CHCl₃)	white solid	diethyl ether	86-88°C
38	(-)-1-(3,5-dichlorophenyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline $[\alpha]_D^{20}$ = -138.3° (c = 1.49, CHCl₃)	white solid	diethyl ether	101-103°C
39	(-)-1-(3,5-dichlorophenyl)-2-cyano-6-methoxy-1,2,3,4-tetrahydroisoquinoline $[\alpha]_D^{20}$ = -101.6° (c = 0.74, CHCl₃).	yellowish amorphous solid	--	--
40	(-)-1-(3,4,5-trimethoxyphenyl)-2-acetyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline $[\alpha]_D^{20}$ = -190.8° (c = 1.02, CHCl₃)	white solid	methanol	119-123°C

BIOLOGICAL DATA

Cell growth inhibition study on human cancer cell lines
Jurkat, MCF-7 and SK-MEL 28

    MCF-7 and SK-MEL 28 cells (~5000 cells/100 µl) were
5   transferred into 96 well plates and grown, with or without
    the test compounds, for 48 hours at 37°C in RPMI medium
    (Gibco) supplemented with 10 % fetal calf serum containing
    penicillin and streptomycin (Gibco). The same procedure was
    followed for Jurkat cells, except for the density of cells
10 (~50000 cells/100 µl) and that the incubation time was lim-
    ited to 24 hours. At the end of the incubation times, the
    cell growth inhibition of the Jurkat and SK-MEL 28 cell lines
    were determined by the use of CellTiter 96 (Promega) and MCF-
    7 with a methylene blue test. The compounds of the examples
15 were found to have in the above tests an IC_{50} of from 8 mi-
    crogram/ml to 150 picogram/ml in at least one cell line.

<u>Cell death by apoptosis</u>

Jurkat and SK-MEL 28 cells were incubated with the compound (I) from example 3 for 6, 24 and 48 hours, after which the percentage of apoptotic cells were determined by Annexin V staining. The results are depicted in the Table 3 below.

Table 3

	Jurkat		SK-Mel-28		
	6 hours	24 hours	6 hours	24 hours	48 hours
vehicle	8	9	12	8	9
compound 3, 500 ng/ml	8	89	8	16	70
SuperFasL, 1000 ng/ml	48	84	8	22	27

The numbers depicted in the Table represent the percentage of Annexin-V positive cells.

From the results in Table 3 above it is obvious that the compound from example 3 induces apoptosis in the tested cell lines, but with a slower kinetics than SuperFasL.

<u>Interaction with cell division</u>

The mitotic index was determined after incubation of SK-Mel-28 cells with vehicle, the compound (I) of example 3 and nocodazole for four hours [essentially as described by C.L. Rieder et al.: Current Biology 10(2000) 1067-1070]. The results are given in table 4 below.

Table 4

	mitotic index SK-Mel-28
vehicle	1.7
compound of example 3, 300 ng/ml	5
Nocodazole, 10 µM	2.9

The tested substances block the cells in the prophase stage
of mitosis.

Inhibition of phosphorylation of IGF-1R and insulin re-
ceptor (IR) in SK-MEL-28

IGF-1R: Assay without treatment with compounds (I).
(Essentially as described by M. Rubini et al. Exp. Cell Res.
230(1997) 284-292).

SK-MEL-28 cells (density 60000/cm^2; 100 mm diameter
dish containing 10 ml RPMI 1640) were starved for 24 hours at
37°C and thereafter treated for 5 minutes at 37°C with IGF-1
(200 ng, Sigma). Untreated cells served as control. The cells
were lyzed and subjected to immunoprecipitation using a spe-
cific antibody against IGF-1R (alfa-IR3, Oncogene Science).
Immunoprecipitates were separated by polyacrylamide gel elec-
trophoresis and transferred to nitrocellulose membranes (Am-
ersham Bioscience). The immunoprecipitated IGF-1 receptor was
located on the nitrocellulose membrane using an antibody
against the alfa-subunit of the IGF-1R (N-20:sc-712, Santa
Cruz Biotech.). Detection of the tyrosine phosphorylation of
the IGF-1 receptor was performed by incubation of the nitro-
cellulose membranes with an anti-phosphotyrosine antibody
(4G10, Upstate Biotechnology Ltd., UK). To reveal the anti-
IGF-1R rabbit polyclonal antibody and the anti-phosphotyro-

sine mouse monoclonal antibody, the membranes were incubated
with anti-rabbit IgG and anti-mouse IgG antibodies coupled to
HRP, respectively, and visualized by the use of an enhanced
chemiluminescence (ECL) detection system (Pierce).

IGF-1R: Assay with treatment with compounds (I).

Starved SK-MEL-28 cells (60000 cells/cm^2; 100 mm diame-
ter dish; containing 10 ml RPMI 1640) were treated for 2
hours with 10 microgram of compound 31. After 2 hours of
treatment, the cells were stimulated for 5 minutes at 37°C
with 200 ng of IGF-1, and thereafter treated as described
above.

Table 5. Percentage of IGF-1R phosphorylation in SK-MEL-28
cells.

Compound 31	0 ng/ml	0 ng/ml	1000 ng/ml
IGF-1	0 ng/ml	20 ng/ml	20 ng/ml
% phosphorylation	0	100	10

IR: Assay with and without treatment with compounds
(I).

SK-MEL-28 cells (density 60000/cm^2) were grown in 100
mm diameter dishes containing 10 ml RPMI 1640 supplemented
with 10% fetal bovine serum (FBS) for 24 hours. After 24
hours fresh medium supplemented with 10 % FBS was added to-

gether with or without 1 microgram/ml of compound 33. The
dishes were incubated at 37°C for 2 hours, after which the
cells were lyzed and subjected to immunoprecipitation using 2
microliter of an anti-IR monoclonal antibody (18-44, ABCAM)
and 20 microliter of agarose-conjugated protein G. Antibody-
antigen complexes were allowed to form for 4 hours at 4°C and
after that collected by centrifugation at 4°C for 1 minute at
5000 rpm. Immunoprecipitated complexes were separated by
electrophoresis on an 8% polyacrylamide gel and electroblot-
ted onto a nitrocellulose membrane (Amersham Bioscience). The
efficiency of the immunoprecipitation was determined by using
a polyclonal antibody against the beta-subunit of the insulin
receptor (C-19, Santa Cruz Biotech.). Detection of the tyro-
sine phosphorylation of the insulin receptor was performed by
incubation of the nitrocellulose membranes with an anti-phos-
photyrosine antibody (4G10, Upstate Biotechnology Ltd., UK).
To reveal the anti-IR rabbit polyclonal antibody and the
anti-phosphotyrosine mouse monoclonal antibody, the membranes
were incubated with anti-rabbit IgG and anti-mouse IgG anti-
bodies coupled to HRP, respectively, and visualized by the
use of an enhanced chemiluminescence (ECL) detection system
(Pierce).

No difference in phosphorylation of the insulin recep-
tor was detected between untreated cells and cells treated
with 1 microgram/ml of compound 33.